Supplementary Data

Effect of flexible linker length on the activity of fusion protein 4-coumaroyl-CoA ligase::stilbene synthase

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Supplementary Table 1. Oligonucleotides used as primers for the PCR amplifications in this study.

Name	Sequence (5'-3')
4CL-up	TAAAGGATCCATGGCGCCACAAGAACAA
4CL-dw	GCCACTCGAGTCACAATCCATTTGCTAG
STS-up	ATCAGGATCCATGGCAGCTTCAACTGAAGAGA
STS-dw	AGTACTCGAGTTAAATGATGGGCACATTCGT
linker-up	ATTTGGATCCATGGCGCCACAAGAACAA
linker-dw	TGATCTCGAGTTAAATGATGGGCACACT
linker-free-inter-end	TCATCTCTTCAGTTGAAGCTGCCATCAATCCATTTGCTAGTTTTGCCCTC
linker-free-inter-star	GAGGGCAAAACTAGCAAATGGATTGATGGCAGCTTCAACTGAAGAGATGA
(GSG)$_1$-inter-end	TCATCTCTTCAGTTGAAGCTGCCATGCCAGATCCCAATCCATTTGCTAGTTTTGCCCTCA
(GSG)$_1$-inter-star	GAGGGCAAAACTAGCAAATGGATTGGGATCTGGCATGGCAGCTTCAACTGAAGAGATGA
(GSG)$_2$-inter-end	TCTCTTCAGTTGAAGCTGCCATGCCAGATCCGCCAGATCCCAATCCATTTGCTAGTTTTGCC
(GSG)$_2$-inter-star	GGCAAAACTAGCAAATGGATTGGGATCTGGCGGATCTGGCATGGCAGCTTCAACTGAAGAGA
(GSG)$_3$-inter-end	CAGTTGAAGCTGCCATGCCAGATCCGCCAGATCCGCCAGATCCCAATCCATTTGCTAGT
(GSG)$_3$-inter-star	ACTAGCAAATGGATTGGGATCTGGCGGATCTGGCGGATCTGGCATGGCAGCTTCAACTG
(GSG)$_4$-inter-end	GTTGAAGCTGCCATGCCAGATCCGCCAGATCCGCCAGATCCGCCAGATCCCAATCCATTTGCTAG
(GSG)$_4$-inter-star	CTAGCAAATGGATTGGGATCTGGCGGATCTGGCGGATCTGGCGGATCTGGCATGGCAGCTTCAACT
(GSG)$_5$-inter-end	GTTGAAGCTGCCATgccagatccgccagatccgccagatccgccagatccgccagatccCAATCCATTTGCT
(GSG)$_5$-inter-star	CTAGCAAATGGATTGggatctggcggatctggcggatctggcggatctggcggatctggcATGGCAGCTTCAACT
(GGGGS)$_1$-inter-end	TCTCTTCAGTTGAAGCTGCCATCGAGCCACCGCCACCCAATCCATTTGCTAGTTTTGCC
(GGGGS)$_1$-inter-star	GGCAAAACTAGCAAATGGATTGGGTGGCGGTGGCTCGATGGCAGCTTCAACTGAAGAGA
(GGGGS)$_2$-inter-end	CAGTTGAAGCTGCCATCGAGCCACCGCCACCCGAGCCACCGCCACCCAATCCATTTGCTAGT
(GGGGS)$_2$-inter-star	ACTAGCAAATGGATTGGGTGGCGGTGGCTCGGGTGGCGGTGGCTCGATGGCAGCTTCAACTG
(GGGGS)$_3$-inter-end	AGTTGAAGCTGCCATcgagccaccgccacccgagccaccgccacccgagccaccgccaccCAATCCATTTGCT
(GGGGS)$_3$-inter-star	CTAGCAAATGGATTGggtggcggtggctcgggtggcggtggctcgggtggcggtggctcgATGGCAGCTTCAACT
(GGGGS)$_4$-inter-end	AGTTGAAGCTGCCATcgagccaccgccacccgagccaccgccacccgagccaccgccacccgagccaccgccaccCAATCCATTTGCT
(GGGGS)$_4$-inter-star	CTAGCAAATGGATTGggtggcggtggctcgggtggcggtggctcgggtggcggtggctcgggtggcggtggctcgATGGCAGCTTCAACT

Note: The underlined sequences in the 4CL-up, STS-up, and linker-up primers indicate the *Bam*HI site. The underlined sequences in the 4CL-dw, STS-dw, and linker-dw primers indicate the *Xho*I site. The underlined sequences in the other primers indicate the nucleotide sequences of the different linkers.
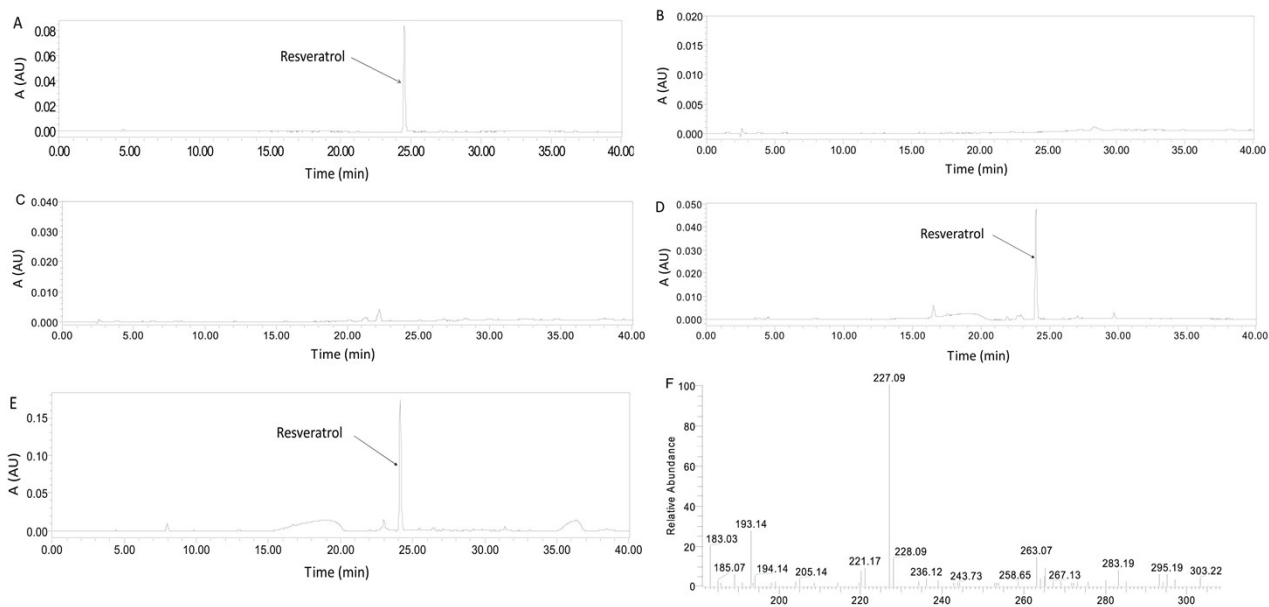
Supplementary Fig. 1. HPLC analysis of resveratrol produced in *S. cerevisiae* and *E. coli*. (A) HPLC profiles (A_{306nm}) of the authentic standard of resveratrol. (B, C) HPLC profiles of culture media from *S. cerevisiae* and *E. coli* expressing 4CL or STS alone, produced no resveratrol. (D) HPLC profiles of culture media from *S. cerevisiae* transformed with 4CL-linker-STS. (E) HPLC profiles of culture media from *E. coli* transformed with 4CL-linker-STS. Peaks indicated are the products of resveratrol. (F) Identification of resveratrol by LC-MS.
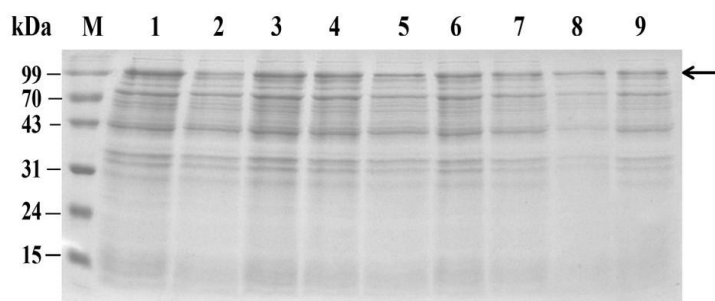
Supplementary Fig. 2. SDS-PAGE analysis of the nine total proteins from the cell lysate after incubation 48 h. Lane 1-5: the total proteins from engineered *E. coli* expressing 4CL-(GSG)$_n$-STS (n≤5), Lane 6-9: the total proteins from engineered *E. coli* expressing 4CL-(GGGGS)$_n$-STS (n≤4). Target protein is indicated by arrow.